Variation in the Hydrocarbon Composition of Non-Africanized Apis mellifera L. Sting Apparatus

By

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ABSTRACT

Sixty-three hydrocarbons were quantitatively measured from 113 individual U.S. commercial stock *Apis mellifera* workers representing 5 age groups and 3 source colonies. Analysis of variance indicated that the abundances of only 3 of the 63 hydrocarbons was unaffected by either age or colony source. The implications of this variability when these hydrocarbons are used for chemotoxonomic investigations are discussed.

INTRODUCTION

Considerable efforts are being expended by several research groups to develop a feasible method for detecting the presence of Africanized genotypes in European colonies of *Apis mellifera*. Approaches currently being tried include morphometric analysis (Daly and Balling 1978, Rinderer et al. 1986), electrophoretic analysis (Daly and Balling 1978, Nunamaker and Wilson 1981, Rinderer and Sylvester 1981, Sylvester 1982) and analysis of cuticular or sting apparatus hydrocarbons (Carson and Bolten 1984, Francis et al. 1985, McDaniel et al. 1984). A lucid analysis of potential pitfalls in these techniques is presented by Page and Erickson (1985).

The critical element in any of these techniques is to assess the range of phenotypic expression for each subspecies and to

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determine how these phenotypes will change when crossbreeding occurs between the various genotypes. Honey bee queens are known to be inseminated by as many as 20 drones (Taber 1958), so that the progeny of a single queen consists of up to 20 half-siblings, fathered by a different haploid drone producing identical semen. Ideally, any technique for detecting Africanized genes will be able to detect the contribution of even a single Africanized male.

In our earlier paper (McDaniel et al. 1984), we qualitatively and quantitatively compared the feasibility of using honey bee hydrocarbons from either the cuticle, sting apparatus, or sting shaft as chemotaxonomic characters. We concluded that sting apparatus hydrocarbons were the more suitable because they possessed the largest number of components (characters), were the least susceptible to environmental contamination, and were present in sufficient quantity to permit quantitative analysis from single bees. Several other parameters needed to be investigated before the reliability of hydrocarbons as indicators of honey bee genotypes could be satisfactorily evaluated. In this paper, we detail the extent of sting apparatus hydrocarbon variation among individual adult non-Africanized honey bees as a function of age and colony origin.

METHODS AND MATERIALS

Insects

Bees were obtained from four sympatric colonies of U.S. commercial stock *A.mellifera* in standard Langstrough hives located in Baton Rouge, Louisiana. Bees were categorized into 5 age categories based on behavioral activities: newly emerged (EM), brood tenders (BR), honeycomb (HC), guard (GD) and foragers (FG) (Free 1965, Ribbands 1952). Foragers were randomly captured in plastic bags as they exited the colony. All others were randomly removed in a similar manner from appropriate locations inside the hive after observation indicated that they were members of a particular behavioral cohort. Seven bees per age group per colony were collected. Sample bees were individually killed by freezing and were stored at -20°C until their sting apparatus could be dissected.

Isolation and quantitation of hydrocarbons

The sting apparatus of each bee was removed from the body with forceps, immersed in pesticide-grade methylene chloride, and stored in a refrigerator prior to sample cleanup and analysis.

After removal of methylene chloride at ambient temperature with a gentle stream of nitrogen, extractable lipids were dissolved in hexane and placed on a Biosil-A[5] minicolumn prepared in a Pasteur pipet. The hydrocarbons were eluted with ca.3 ml hexane and the volume again reduced to near dryness with a gentle stream of nitrogen. The residues were then dissolved in 0.25 ml carbon disulfide and analyzed by capillary gas chromatography (GC) utilizing a flame ionization detector (FID).

Gas chromatographic analyses were performed with a Perkin-Elmer Sigma 115 gas chromatograph and data station. A 30m by 0.32mm DB-5 column (J&W Scientific, Incorporated) was utilized in the splitless mode for the separation with the following gas chromatographic parameters: Injection port temperature —280°C, FID detector temperature —330°, oven temperature —initially 125° (hold 6 minutes), then programmed to 305° at 10° per minute (hold 11 minutes at 305°). The carrier gas was ultra pure helium at a head pressure of 210 kPa. Data were then transferred to a floppy disk with a Perkin-Elmer computer. Equivalent chain lengths of individual components were calculated from retention times of n-alkane standards and were used to associate individual GC peaks with previously identified components that had been separated by packed column gas chromatography.

Data analysis

Least squares analyses of variance were conducted for a two factor linear model which utilized colony source, age of the bees and interactions. Linear contrasts were used to separate least squares means. Data were expressed as proportions and transformed to the arc sine of the square root of proportions for tests of hypotheses. Reported means and coefficients of variation (CV's) are untransformed data. Twenty-seven of the 140 samples, distributed over all age groups except foragers, were lost in a laboratory accident.

RESULTS AND DISCUSSION

Sixty-three hydrocarbon peaks distinguishable by capillary GC were chosen for examination (Table 1). Using means based on all samples (n = 113), 27 peaks (42%) were present in abundances greater than 1%. An additional 9 peaks (14%) were present

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[5]Mention of a company or a trade name is for identification purposes only and does not imply endorsement by the U.S. Department of Agriculture.

in abundances greater than 0.5%, and the remaining 28 peaks (44%) were present in trace quantities (<0.5%). Table 1 presents the mean proportion data (averaged over all samples) for each component and their associated coefficients of variation. For the 36 components with mean abundances greater than 0.5%, only 6 (component numbers 6,9,11,14,18,29) were shown by ANOVA to be unaffected by either source or age. Of these 6, however, 3 (numbers 6,9,14) have CV's that range from 101.6% to 726%. While these 3 peaks are statistically similar, they are not likely to be taxonomically useful because of the high variances[6]. The 28 components present in trace quantities likewise show large CV's and taxonomically are probably not useful.

The abundance of 30 of the peaks present in larger than trace quantities were shown by ANOVA to be either age related (Table 2), colony related (Table 3), or both age and colony related (Table 4). Although cause-effect relationships between age and colony source patterns for the sting apparatus hydrocarbons are not clear at this time, our observations on newly emerged bees are consistent with those of Blomquist et al. (1980) and Francis et al. (1985); both noted that the cuticular hydrocarbons of newly emerged bees are most different from those of other age categories, regardless of source colony.

Table 5 presents the ranges of abundance for 8 olefinic sting components. Carlson and Bolten (1984) and Francis et al. (1985) suggested that these olefins (as cuticular components) may be used as characteristic phenotypic markers for Africanized genotypes of *A. mellifera*. The sting data presented in Table 5 are *from commercial U.S. stocks of A. mellifera* containing no known Africanized genotypes and all 8 compounds are clearly present in percent abundances approaching those reported in Africanized bees.

The 63 hydrocarbon components shown in Table 1 possessed an unexpected degree of variability associated with the ages of the bees and the source colonies. A similar variability will likely be found to exist with the cuticular hydrocarbons of these bees. This problem may be partly ameliorated by sampling from a single age cohort (foragers, for example) but inter-colony variation will remain. An alternative would be to pool extracts from several bees, providing an "average" phenotypic profile for that particular colony. Using such an average is not without its dangers, however.

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[6]Mean proportions of all components vary by 6.65max/0.04min x 100 = 16,600%. Thus, even those coefficients with CV >700% could be useful taxonomically.

Table 1. Mean percent composition and coefficient of variation of the hydrocarbons from the stings of *Apis mellifera* L.

GC* peak	Equivalent chain length	Mean percent	Average CV** of mean percent
1	17.95	0.1	89.9
2	18.00	0.04	102.1
3	18.72	1.24	124.5
4	19.00	0.45	126.1
5	19.35	1.20	98.0
6	19.72	2.26	151.5
7	20.00	0.35	79.1
8	20.34	0.21	241.1
9	20.73	1.14	725.7
10	21.00	1.27	47.1
11	22.00	1.48	56.8
12	22.72	0.85	151.4
13	23.00	5.06	52.2
14	23.23	2.38	101.6
15	23.36	1.94	156.5
16	23.56	0.20	97.0
17	23.73	0.13	137.2
18	24.00	3.02	60.5
19	24.62	0.90	98.9
20	24.71	0.14	181.2
21	25.00	5.87	44.8
22	25.32	0.24	146.4
23	26.00	2.77	56.3
24	26.72	0.61	69.3
25	27.00	6.65	36.9
26	27.31	0.73	63.0
27	27.56	0.21	68.0
28	27.70	1.40	89.6
29	28.00	2.78	40.3
30	28.31	1.60	203.3
31	28.76	0.94	55.5
32	29.00	5.93	33.3
33	29.31	1.02	94.7
34	29.59	0.58	91.5
35	29.78	0.26	92.1
36	30.00	2.80	37.1
37	30.58	0.30	86.5

Table 1. Mean percent composition and coefficient of variation of the hydrocarbons from the stings of *Apis mellifera* L. (continued)

GC peak	Equivalent chain length	Mean percent	Average CV of mean percent
38	30.80	3.07	64.5
39	31.00	4.98	35.9
40	31.28	0.46	97.2
41	32.00	2.02	38.1
42	32.56	0.51	103.3
43	32.83	4.00	68.3
44	33.00	2.06	44.0
45	33.28	0.24	158.4
46	34.00	1.71	46.0
47	34.59	0.49	213.8
48	34.81	0.78	71.5
49	35.00	1.09	51.1
50	36.00	1.15	57.9
51	36.53	0.58	83.2
52	36.72	0.17	171.2
53	37.52	0.30	151.2
54	37.81	0.46	81.7
55	38.49	0.33	78.0
56	38.63	0.09	160.4
57	39.26	0.14	171.7
58	40.41	0.05	216.9
59	40.63	0.23	130.9
60	≅ 41.60‡	0.09	156.8
61	≅ 43.39‡	0.08	280.5
62	≅ 43.63‡	0.08	142.0
63	≅ 43.88‡	0.06	271.8

*GC = gas chromatographic.

**n = 113; CV = coefficient of variance.

‡Extrapolated values outside the range of our hydrocarbon standards.

Table 2. Mean percent abundance and associated coefficient of variation for sting apparatus hydrocarbon components shown by ANOVA to be age dependent.

Age*	GC** peak number					
	3	25	32	33	39	43
EM	0.21 a	4.22 a	3.77 a	1.89	3.31 a	0.65 a
BR	0.81 a	7.21 bc	6.16 bc	0.92 a	4.93 b	4.84 b
HC	1.33 ab	5.90 b	5.47 bc	1.00 a	4.87 b	4.94 b
GD	1.55 ab	7.58 bc	6.72 c	0.71 a	5.53 b	5.45 b
FG	1.92 b	7.76 bc	6.90 c	0.79 a	5.77 b	3.77 b
Mean (%CV)	1.24(124.5)	6.65(36.9)	5.93(33.3)	1.02(94.7)	4.98(35.9)	4.00(68.3)

*Newly emerged (EM), brood tenders (BR), honeycomb (HC), guard (GD), foragers (FG).

**Gas chromatographic values are taken from Table 1.

Column means followed by the same letter are not significantly different at $\alpha = 0.05$.

Table 3. Mean percent abundance and associated coefficient of variation for sting apparatus hydrocarbon components shown by ANOVA to be colony dependent.

Colony	GC* peak number									
	30	41	44	46	48	49	50	56	58	59
1	0.95 a**	1.52 a	1.65 ab	1.08 a	0.98 b	0.23 a	0.44 a	0.13 b	0.00 a	0.31 b
2	0.63 a	1.75 a	2.01 ab	1.77 b	0.77 a	1.40 b	1.42 b	0.05 a	0.05 a	0.04 a
3	3.98 b	1.55 a	1.50 a	1.10 a	1.91 c	0.26 a	0.57 a	0.10 b	0.01 a	0.43 b
4	1.07 a	3.28 b	3.07 c	2.88 c	0.55 a	2.42 c	2.13 c	0.08 b	0.13 b	0.13 a
Mean (%CV)	1.60(203)	2.02(38)	2.06(44)	1.71(46)	0.78(72)	1.09(51)	1.15(58)	0.09(160)	0.05(217)	0.23(131)

*Gas chromatographic values taken from Table 1.
**Column means containing the same letter are not significantly different at = 0.05.

Table 4. Mean percent abundance and associated coefficient of variation for sting apparatus hydrocarbon components shown by ANOVA to be both age and colony dependent.

Colony	Age*	GC** peak number					
		10	13	21	38	51	55
1	EM	2.19	8.20	4.44	0.39	2.13	0.99
1	BR	1.35	6.25	6.97	2.93	0.47	0.33
1	HC	1.87	9.08	8.09	3.70	0.75	0.43
1	GD	0.79	5.13	6.97	5.78	0.44	0.29
1	FG	1.39	4.84	6.87	4.11	0.58	0.48
2	EM	2.02	5.16	3.21	0.27	0.48	0.09
2	BR	1.02	3.19	5.83	4.48	0.55	0.12
2	HC	1.02	2.22	3.33	5.26	0.24	0.03
2	GD	1.13	4.42	6.18	2.75	0.36	0.04
2	FG	0.81	6.90	7.70	3.25	0.21	0.08
3	EM	3.03	7.24	3.35	0.24	1.44	0.95
3	BR	0.90	5.18	7.34	5.47	0.70	0.42
3	HC	1.27	2.96	3.20	2.71	0.99	1.08
3	GD	0.90	5.09	8.83	5.23	0.60	0.40
3	FG	1.29	7.13	8.27	3.37	0.82	0.62
4	EM	1.66	6.62	6.68	0.49	0.32	0.03
4	BR	0.89	2.94	3.99	1.81	0.28	0.15
4	HC	1.41	4.19	3.48	2.05	0.22	0.08
4	GD	0.91	2.69	4.94	3.12	0.39	0.15
4	FG	0.69	3.15	5.34	2.60	0.22	0.06
Mean (%CV)		1.27 (47)	5.06 (52)	5.87 (49)	3.07 (65)	0.58 (85)	0.33 (78)

*Bees were categorized into five approximate age groups based on behavioral activities: newly emerged (EM), brood tenders (BR), honeycomb (HC), guard (GD), foragers (FG).

**Gas chromatographic values are taken from Table 1.

Table 5. Range of percent abundance for olefinic components with more than 34 carbons in random-age and forager-only samples of U.S commercial stock *Apis mellifera* sting hydrocarbons.

Component*	GC peak number**	Range in percent abundance	
		Random age‡	Foragers‡‡
35:2	47	0 - 10.95	0 - 0.98
35:1	48	0 - 4.17	0.16 - 1.29
37:2	51	0 - 4.45	0 - 1.72
37:1	52	0 - 2.16	0 - 1.07
39:2	55	0 - 2.33	0 - 1.49
39:1	56	0 - 0.98	0 - 0.45
41:2	58	0 - 0.65	0 - 0.52
41:1	59	0 - 1.82	0 - 1.82

*Carbon number: number of double bonds.
**Gas chromatographic values are taken from Table 1.
‡Based on 113 bees from 5 age groups and 4 colonies.
‡‡Based on 28 bees from 4 colonies.

Even if different genotypes (such as those of the Africanized bee) do indeed have distinctive GC profiles, the ability to recognize the phenotypic expression of a rare genotype in a pooled sample will depend on a combination of the relative abundance of all genotypes and associated variability of expression of those characters by individual bees. Because no unique phenotypic hydrocarbon marker for any of the genotypes of *A. mellifera* has yet been found (Table 5), the problem of detecting variable proportions of individual honey bee genotypes in a colony is probably best approached by using carefully designed sampling protocols in conjunction with the judicious application of multivariate discriminant analysis techniques to the resulting hydrocarbon character sets. Such a procedure has been applied by us to Africanized and non-Africanized South American populations of *A.mellifera* and will be the subject of a forthcoming paper.

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